REMARKS

Applicant thanks the Examiner for the courtesy of the telephone interview on March 28, 2001. Applicant has amended claims 1, 2, 20, 21 to recite that the binding pair is in a binding pair complex. Support for this amendment can be found throughout the specification, including at page 4, lines 15-16, page 6, lines 12-21, and Figure 1. Applicant respectfully requests reconsideration and allowance of claims 1-21 in view of the above amendment and following remarks.

Applicant acknowledges entry of the amendment after final, submitted December 11, 2000, and withdrawal of the objections to the specification. Applicant request that initialed copies of the Form 1449s submitted with the Information Disclosure Statement of January 22, 2001, and the Information Disclosure Statement submitted herewith, be returned in the next communication from the Patent Office, indicating that the cited references have been considered.

Rejections under 35 U.S.C. § 103

The Examiner maintained the rejection of claims 1-21 under 35 U.S.C. §103(a) as being unpatentable over Kortright, et al. (U.S. Patent No. 4,870,003) in view of Jackson, et al. (U.S. Patent No. 5,776,709). The Examiner characterized Kortright et al. as disclosing "a solid-phase immunoassay for the simultaneous detection of both members of a binding pair in physiological fluid through the utilization of labeled antibodies with specific binding affinities for said binding pair members." Kortright et al. also was characterized as disclosing "methods consisting of coating a solid phase reagent with a capture antibody (anti-HIV monoclonal antibody); exposing said solid phase reagent to a biological sample to bind one member of the binding pair; and adding labeled antibodies to detect the levels of each member of the binding pair" and noted column 4, lines 1-32 of Kortright et al. Jackson et al. was characterized as disclosing the "use of fluorescently labeled antibodies" and "methods for using multiple stains simultaneously in flow cytometry." The Examiner also contended that "it would have been obvious to one of skill in the art to use the fluorochrome labeling disclosed by Jackson et al. in the methods disclosed by Kortright et al. in order to reap the benefits of direct measurement (both qualitative and quantitative) of each label (and hence each binding pair member) as well as the reduction in sample preparation and data acquisition. One would have a reasonable expectation of success

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since Kortright et al. suggest the use of other labeling systems, specifically 'fluorescers' (see column 7 lines 29-32)." Applicant respectfully traverses.

Independent claim 1, as amended, recites a method for simultaneously measuring both members A and B in a binding pair complex (i.e., the method is capable of detecting members A and B when bound to each other) in a biological sample, while amended independent claim 20 recites a kit for simultaneously measuring both members A and B in a binding pair complex. The method includes:

a) providing a solid phase reagent, the solid phase reagent comprising a particle coated with capture antibodies having specific binding affinities for member A of the binding pair complex;

b) contacting the biological sample with the solid phase reagent under conditions in which member A, if present, becomes bound to the particle, to form a first reacted particle;

c) contacting the first reacted particle with first antibodies having specific binding affinities for member A, wherein the first antibodies are labeled with a first label, and with second antibodies having specific binding affinities for member B of the binding pair complex, wherein the second antibodies are labeled with a second label, to form a second reacted particle, and

d) measuring the first and second labels on the second reacted particle using flow cytometry.

The present claimed invention provides a rapid and sensitive method that can be used to enhance the ability to detect infection or other pathologies at an early stage, leading to earlier treatments. The art has long sought a method for detecting infection such as HIV or other pathologies at an early stage, leading to earlier treatments. In an article published in 1998, Constantine indicated (reference AR of the Form 1449 submitted herewith) that simultaneous detection of HIV-1 antigen p24 and host antibody in a binding pair complex had not been achieved. See, for example, page 2 of the Constantine reference, which indicates that when antibodies to HIV become detectable, "p24 antigen is often no longer demonstrable" and that this is "most likely due to antigen-antibody complexing in the blood." Constantine also indicates that detection of p24 antigen bound to anti-p24 antibody requires pretreatment with an acid to dissociate the complex. See, page 2 of the Constantine reference. Even a package insert for an

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HIV-1 p-24 antigen assay from Coulter Corporation, the assignee of the Kortright et al. patent, indicates that the assay is for the detection of uncomplexed p24 antigen. See, page 1 of reference AQ of the Form 1449 submitted herewith. These references evidence the long-felt but unmet need for the ability to simultaneously detect both members A and B in a binding pair complex, as recited in the present claims.

As the Examiner knows and as indicated in MPEP §2141, the following tenets of patent law must be adhered to when applying an obviousness rejection: "(A) The claimed invention must be considered as a whole; (B) The references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination; (C) The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention and (D) Reasonable expectation of success is the standard with which obviousness is determined. Hodosh v. Block Drug Co., Inc., 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986)."

The combination of the Kortright et al. and Jackson et al. references does not teach or suggest the presently claimed methods or kits. The Kortright et al. reference discloses a system for detecting HIV antigen and anti-HIV antibody that includes capturing viral antigen with an immobilized anti-HIV antibody and detecting with a biotin-labeled, human, anti-HIV antibody. A known concentration of viral antigen ("the spike") is added to the patient's sample prior to the assay. The Jackson et al. reference discloses a flow cytometry method for analyzing populations of leukocytes that uses two or more fluorescent labels. Leukocytes are identified by combinations of cell surface markers.

In particular, the combination of the Kortright et al. and Jackson et al. references does not teach or suggest that two antibodies, one having specificity for member A and one having specificity for member B, that are differentially labeled can be used to simultaneously detect both members in a binding pair complex. In contrast, the presence of one of the members of a binding pair is determined indirectly in the Kortright et al. reference by spiking the samples with viral antigen and noting if the spiked antigen increases or decreases optical density. See, column 3, lines 42 - 61 of the Kortright et al. reference, which explain the rational for the assay. The assay of the Kortright et al. reference spikes a known concentration of virus antigen into the serum or plasma sample. If free antibody is present in the sample, the antibody will bind to the

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spiked viral antigen and will interfere with the detection of the spiked viral antigen. If less than the known amount of antigen is detected, the sample is construed to be "antibody positive". If the antigen spike has an additive effect with the sample, the sample is construed to be "antigen positive". If the concentration of the spiked sample is measured to be the same as the known amount of the spike, then the sample is construed to be "negative for both antibody and antigen". The underlying premise of the Kortright et al. reference is that host antibody present in the sample will interfere with the results of the assay. Consequently, any antibody present in the sample will interfere with the actual quantitation of the analyte in that assay system and the assay can do no more than determine if there is "free" antigen present or "free" antibody (free meaning uncomplexed with antibody, antigen, or other interfering substance). In other words, the assay of the Kortright et al. reference is unable to detect an immune complex of virus and antibody. Thus, the Kortright et al. reference is not simultaneously measuring both members A and B in a binding pair complex as recited in independent claims 1 and 20.

In contrast to the assay of Kortright et al., the present assay allows both members in a binding pair complex to be directly and simultaneously measured since, with respect to the present claims, the first antibody and member B (e.g., host antibody) do not functionally interfere with each other's binding to member A. Step C of amended claim 1 recites that a second reacted particle is formed by contacting the first reacted particle (capture antibody and member A) with a first labeled antibody and a second labeled antibody. The first reacted particle is formed in the presence or absence of member A. To form the second reacted particle, the first antibody must be able to bind member A without functional interference from member B. This is exactly the opposite of the underlying premise of the assay disclosed in the Kortright et al. reference, which is based on interference by host antibody.

Furthermore, the Kortright et al. reference is assuming that it is host antibody present in the sample that interferes with the results of the assay. Applicant notes, however, that a variety of proteins can interfere with results of the assay. For example, with respect to HIV, soluble chemokine receptors or soluble CD4 also can bind to antigen and interfere with the results of the assay. Thus, in contrast to the presently claimed methods and kits, the identity of member B of the binding pair cannot be unambiguously identified in the Kortright et al. reference.

The Jackson et al. reference does not remedy the deficiencies of the Kortright et al. reference, as the Jackson et al. reference does not teach or suggest that both members A and B in a binding pair complex can be measured simultaneously. In fact, the Jackson et al. reference does not even measure a binding pair. Subpopulations of leukocytes are measured with antibodies that bind to different cell surface markers. Using multiple fluorescent markers in the assay disclosed in the Kortright et al. reference still does not allow one to measure both members in a binding pair complex. Again, the assay disclosed in the Kortright et al. reference only indirectly infers if antibody is present and does not allow one to simultaneously measure both members in a binding pair complex. Applicant submits that the present claims are non-obvious. In view of the above, the Examiner is requested to withdraw the rejection under 35 U.S.C. §103.

CONCLUSION

Attached is a marked-up version of the changes being made by the current amendment. Applicant requests reconsideration and allowance of claims 1-21. The Examiner is invited to telephone the undersigned agent if it is felt that such would advance prosecution of the application.

Enclosed is a request for extension of time with the required fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 4/6/01

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Version with markings to show changes made

In the claims:

Claims 1, 2, 20, and 21 have been amended as follows:

1. (Twice Amended) A method for simultaneously measuring both members A and B [of] in a binding pair complex in a biological sample, said method comprising:

a) providing a solid phase reagent, said solid phase reagent comprising a particle coated with capture antibodies having specific binding affinities for said member A of said binding pair complex;

b) contacting said biological sample with said solid phase reagent under conditions in which said member A, if present, becomes bound to said particle, to form a first reacted particle;

c) contacting said first reacted particle with first antibodies having specific binding affinities for said member A, wherein said first antibodies are labeled with a first label, and with second antibodies having specific binding affinities for said member B of said binding pair complex, wherein said second antibodies are labeled with a second label, to form a second reacted particle, wherein said first and second labels are different and

d) measuring said first and second labels on said second reacted particle using flow cytometry.

2. (Amended) The method of claim 1, wherein substantially all said capture antibodies are oriented on said particle such that the antigen binding regions of said capture antibodies are available for binding said member A of said binding pair complex.

20. (Twice Amended) A kit for simultaneously measuring both members A and B [of] in a binding pair complex in a biological sample, said kit comprising:

a) a solid phase reagent, said solid phase reagent comprising a particle coated with capture antibodies having specific binding affinities for said member A of said binding pair complex, wherein substantially all said capture antibodies are oriented on said particle such that

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the antigen binding regions of said capture antibodies are available for binding said member A of said binding pair complex;

b) first antibodies having specific binding affinities for said member A of said binding pair complex, wherein said first antibodies are labeled with a first label; and

c) second antibodies having specific binding affinities for said member B of said binding pair complex, wherein said second antibodies are labeled with a second label, and wherein said first and second labels are different.

21. (Amended) The kit of claim 20, said kit further comprising a label or package insert, wherein said label or package insert indicates that said solid phase reagent, said first antibodies, and said second antibodies can be used for simultaneously measuring both members A and B [of] in a binding pair complex in a biological sample by flow cytometry.